Interactions of unsaturated fat or coconut oil with Rumensin® on milk fat production might be mediated through inhibition of specific protozoal genera

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Introduction

Polyunsaturated fatty acids (**PUFA**) feeding often decreases protozoal numbers in the rumen. Animal-vegetable fat (**AV**), a by-product of the food industry, is readily available to provide PUFA in dairy diets. However, the response to AV supplementation on protozoal numbers is not consistent, possibly due to biohydrogenation (**BH**) of PUFA in the rumen. Long chain saturated FA are less toxic to protozoa; therefore, the BH of PUFA removes their potential inhibitory effects. In contrast, evidence from OSU supports the contention that protozoa are a vehicle for passage of PUFA or other intermediates of BH that do not promote MFD.

AV supplementation in combination with Rumensin® (**R**), an ionophore improving feed efficiency, occasionally spontaneously decreases milk fat yield and percentage. This milk fat depression (**MFD**) is likely due to the partial BH of PUFA, which favors FA intermediates that are inhibitory to milk fat synthesis.

Feeding coconut oil (**CO**) rich in medium chain fatty acids (**MCFA**), and therefore low in PUFA, has decreased the abundance of ruminal protozoa in sheep. We hypothesized that, while lowering protozoal populations, diets supplemented with CO in combination with R would not cause MFD as would AV diets combined with R. PUFA or MCFA in combination with R could shift ruminal fermentation and potentially depress fiber degradation, reducing fed intake.

Objectives

Therefore our objectives were to determine the effects of feeding AV or CO in combination with R on protozoal abundance, ruminal fermentation, total tract digestibility, feed intake, milk and milk fat production. This interaction between R and fat source on MFD is reported here with a 2x3 factorial arrangement of treatments with +/- R and either no fat, AV, or CO.

Material and methods

Six primiparous rumen-cannulated Holstein cows (79 DIM) were fed six diets in a 6x6 Latin square design. The diets were supplemented or not with 260 mg/d of R (+/- R), and with control (no fat added), 5% AV, or 5% CO in a 2x3 factorial arrangement. Periods were 3 wk except the 4-week initial period to allow adequate adaptation of ruminal populations to R; in subsequent periods, rumen contents were transferred to hasten adaptation. Diets were prepared once daily as a TMR and fed every 2 h.

All diets contained 16.2% alfalfa hay and 32.8% corn silage on a DM basis. The diet composition averaged 16.6% CP, 5.5% ash and 41.5% NFC, similar for all diets. Diets averaged 2.4, 5.8, and 6.4% of FA for control, AV, and CO, respectively, and the FA profile is reported in Fig. 1. The diets had 33.6, 29.6, and 28.5 %NDF for control, AV, and CO, respectively. The R was measured and verified within expected ranges in the 3 supplemented diets.

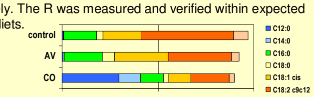

Figure 1. FA profile in % in control, AV and CO diets.

The mixed model included fixed (diet) and random (period, cow) effects. Contrasts were the main effects of: 1) R , 2) Fat supplementation (control vs. AV+CO), and 3) Source of fat (AV vs. CO); and 4 and 5) the interactions of R with contrasts 2 (RxF) and 3 (RxS). Significance was P<0.05 for main effects and P<0.10 for interactions.

Results

- CO ↓ protozoa numbers (Fat, Source, P<0.01, Table 1, Fig. 1)
  - ↓ 93% for total counts
  - ↓ 99% for *Isotricha* and *Dasytricha*
  - ↓ 97% for *Entodinium*
  - ↔ *Epidinium* → resistant to CO
- AV+R ↓ *Epidinium* (RxS, P = 0.02)
  - ↓ 67% with R
  - ↓ 92% with AV+R
  - → sensitive to R and AV

Table 1. LS means of log 10 for total and genera specific protozoan cell counts per ml rumen fluid.[1]

	-R			+R				Contrasts[2]				
	Control	AV	CO	Control	AV	CO	SE	Rum	Fat	Source	RxF	RxS
Total	5.91	5.86	4.86	6.01	5.98	4.75	0.14	NS	<0.01	<0.01	NS	NS
Isotricha	3.89	4.25	2.24	3.90	3.99	1.82	0.40	NS	<0.01	<0.01	NS	NS
Dasytricha	3.30	2.41	0.74	3.81	3.32	1.29	0.50	0.07	<0.01	<0.01	NS	NS
Entodinium	5.85	5.79	4.65	5.96	5.93	4.44	0.17	NS	<0.01	<0.01	NS	NS
Epidinium	2.97	3.73	3.42	2.43	1.88	3.22	0.83	<0.01	NS	0.13	NS	0.02

[1] +/- R: 260 mg/d of Rumensin®, control: no fat added, AV: 5% animal-vegetable fat added, CO: 5% coconut oil added.
[2] Probability of a treatment response; NS = not significant (P > 0.20).

- No change in total VFA concentration (Table 2)
- AV and CO ↓ acetate ↑ propionate (Fat, Source, P<0.01)
- CO ↓ protozoal numbers ↔ ↓ butyrate (Source, P<0.01)

Table 2. LS means for ruminal fermentation.[1]

	-R			+R				Contrasts[2]				
	Control	AV	CO	Control	AV	CO	SE	Rum	Fat	Source	RxF	RxS
Total VFA, mM	133	125	129	144	127	123	5	NS	<0.01	NS	0.12	NS
VFA, mol/100 mol												
Acetate	62.2	60.0	54.7	62.7	57.7	54.4	1.0	NS	<0.01	<0.01	0.13	0.16
Propionate	21.5	23.4	30.2	21.5	24.5	30.5	1.6	NS	<0.01	<0.01	NS	NS
Butyrate	12.4	12.7	10.5	11.7	13.6	11.0	1.0	NS	NS	<0.01	0.17	NS

[1] +/- R: 260 mg/d of Rumensin®, control: no fat added, AV: 5% animal-vegetable fat added, CO: 5% coconut oil added.
[2] Probability of a treatment response; NS = not significant (P > 0.20).

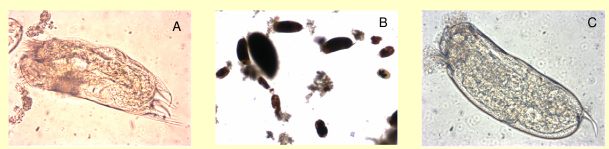

Figure 2. Panel of micrographs of protozoan cells from rumen contents of cows on AV or CO diets.

A. *Ophryoscolex* using phase contrast (400X)
B. Mixed protozoa cells stained with methyl blue and Lugol's reagent (100X)
C. *Epidinium* using phase contrast (400X)

Table 3. LS means of apparent nutrient digestibility of the total tract.[1]

	-R			+R				Contrasts[2]				
	Control	AV	CO	Control	AV	CO	SE	Rum	Fat	Source	RxF	RxS
OM	68.2	68.0	67.7	65.7	70.7	67.3	1.5	NS	NS	NS	0.17	NS
NDF	56.7	46.0	30.5	49.8	47.0	40.5	3.7	NS	<0.01	<0.01	0.04	0.19
FA	69.2	69.5	88.2	72.8	74.7	91.0	4.4	0.16	<0.01	<0.01	NS	NS
Total C18	73.5	68.5	81.0	76.8	75.0	88.7	4.5	0.05	NS	<0.01	NS	NS

[1] +/- R: 260 mg/d of Rumensin®, control: no fat added, AV: 5% animal-vegetable fat added, CO: 5% coconut oil added.
[2] Probability of a treatment response; NS = not significant (P > 0.20).

- ↓ NDF digestibility (Fat, Source, RxF, Table 3)
  - CO ↓ acetate ↔ ↓ NFD digestibility
- CO ↑ FA digestibility (Fat, Source, P<0.01)
  - MCFA more digestible
- +R ↑ C18 FA digestibility (Rum, P=0.05)
  - Incomplete BH, UFA more digestible

Table 4. LS means of dry matter intake and milk production.[1]

	-R			+R				Contrasts[2]				
	Control	AV	CO	Control	AV	CO	SE	Rum	Fat	Source	RxF	RxS
DMI, kg/d	20.0	19.8	15.5	19.3	19.0	14.8	0.7	0.08	<0.01	<0.01	NS	NS
Milk, kg/d	33.9	34.3	30.5	33.1	31.7	30.1	2.0	0.06	<0.01	<0.01	NS	0.16
Milk fat, g/d	1.08	1.01	0.71	1.05	0.87	0.74	0.05	0.15	<0.01	<0.01	NS	0.08

[1] +/- R: 260 mg/d of Rumensin®, control: no fat added, AV: 5% animal-vegetable fat added, CO: 5% coconut oil added.
[2] Probability of a treatment response; NS = not significant (P > 0.20).

- CO ↓ DMI and milk production (Fat, Source, P<0.01, Table 4.)
  - DMI ↓ 5 kg/d
- AV+R and CO ↓ milk yield and fat (Fat, Source, P<0.01)
  - MFD with AV+R (RxS, P=0.08)
  - MFD with CO

Discussion

Feeding CO drastically decreased protozoal cell counts and shifted ruminal fermentation toward propionate at the expense of acetate and butyrate. Although total protozoal counts were not affected by the interaction RxS, the counts of *Epidinium* were lower when fed AV+R. Because this diet also caused MFD, *Epidinium* may be involved in BH mechanisms.

Against our hypothesis, diets supplemented with CO also induced MFD, possibly through another mechanism than AV diets. Indeed, feeding CO differentially affected protozoal genera with no toxic effects on *Epidinium.*

The changes in VFA were associated with a decreased total tract digestibility of NDF for CO from inhibition of fiber degradation in the rumen. This inhibition is associated by the lower DMI with CO from rumen fill. Total tract digestibly of FA was higher with CO due to higher duodenal digestibility of MCFA. Higher C18 digestibility with R could result from more UFA from incomplete BH, causing the MFD observed when AV+R was fed.

Although the inhibition of fiber degradation might have limited energy for milk fat synthesis, further analysis of omasal and milk FA will help elucidate the mechanism of MFD with CO supplementation.

Conclusion

Although MCFA are not a good alternative to PUFA to prevent MFD in dairy cows, their use can help identify the ruminal changes of microbial populations and the mechanisms of BH that promotes MFD. *Epidinium* displayed resistance to MCFA toxicity and may be involved in BH mechanisms.

Abstract

Feeding animal-vegetable (AV) fat or medium chain FA to dairy cows can decrease rumen protozoal count. In contrast, AV fat with Rumensin (R) can promote milk fat depression (MFD), whereas diets supplemented with coconut oil (CO; rich in medium chain FA) + R were not expected to cause MFD. In a 6x6 Latin square design (2x3 factorial), 6 rumen-cannulated cows were fed with +/-R (260 mg/d) and either: control (no fat), 5% AV, or 5% CO. Diets were balanced to have 21.5% forage NDF, 16.8% CP and 42% NFC. The mixed model included fixed (diet) and random (period, cow) effects. Contrasts were the main effects of: 1) R(+R), 2) fat supplementation (control vs. AV+CO, and 3) fat source (AV vs. CO); and 4 and 5) the interactions of R with contrasts 2 and 3. Significance was P<0.05 for main effects and P<0.10 for interactions. The log10 of concentrations of total protozoa (cells/ml) were not different from control (5.97) vs. AV (5.95) but decreased by 93% with CO (4.79). *Isotricha* and *Entodinium* decreased by 99 and 97% by CO, whereas *Epidinium* was unchanged. In contrast, *Epidinium* were 67% lower for the main effect of +R and decreased 92% when AV was supplemented with R. Total VFA concentration (130 mM) was not affected by diet, but the A:P ratio decreased for CO (1.85) vs. control (2.95) or AV (2.58). The low A:P ratio was associated with a decreased total tract digestibility of NDF for CO (35.5%) vs. control (53.3%) and AV (46.5), with no difference in OM digestibility (averaging 67.9%). DMI was 5 kg/d lower with CO (15.3 kg/d) and not different for control and AV. Milk production was lower with +R (31.6 kg/d) and CO (30.3 kg/d) than AV (33.0 kg/d). MFD occurred with AV+R and CO: 1.08, 1.01, 0.71, 1.05, 0.87, and 0.74 kg/d for control, AV, CO, control+R, AV+R, and CO+R, respectively. Further analyses should elucidate the role of protozoal concentration and genera on bacterial biohydrogenation in the rumen.

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